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Position-specific isotope modeling of organic micropollutants transformation through different reaction pathways

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Highlights

- Mechanism-based, position-specific isotope modeling of micropollutants degradation
- Simultaneous description of concentration and primary and secondary isotope effects
- Key features of the model are demonstrated with three illustrative examples
- Model as a tool to explore reaction mechanisms and to design experiments

Abstract

The degradation of organic micropollutants occurs via different reaction pathways. Compound specific isotope analysis is a valuable tool to identify such degradation pathways in different environmental systems. We propose a mechanism-based modeling approach that provides a quantitative framework to simultaneously evaluate concentration as well as bulk and position-specific multi-element isotope evolution during the transformation of organic micropollutants. The model explicitly simulates position-specific isotopologues for those atoms that experience isotope effects and, thereby, provides a mechanistic description of isotope fractionation occurring at different molecular positions. To demonstrate specific features of the modeling approach, we simulated the degradation of three selected organic micropollutants: dichlorobenzamide (BAM), isoproturon (IPU) and diclofenac (DCF). The model accurately reproduces the multi-element isotope data observed in previous experimental studies. Furthermore, it precisely captures the dual element isotope trends characteristic of different reaction pathways as well as their range of variation consistent with observed bulk isotope fractionation. It was also possible to directly validate the model capability to predict the evolution of position-specific isotope ratios with available experimental data. Therefore, the approach is useful both for a mechanism-based evaluation of experimental results and as a tool to explore transformation pathways in scenarios for which position-specific isotope data are not yet available.

Capsule abstract

We propose a modeling approach incorporating mechanistic information and predicting concentration and position-specific isotopic evolution during organic micropollutants degradation.

Keywords: Organic micropollutants, CSIA, isotope modeling, position-specific isotope fractionation, transformation pathways

Introduction

Numerous synthetic organic compounds are produced annually in vast quantities for industrial manufacturing processes, agricultural use, as well as for human and animal healthcare (Fenner et al., 2013; Schwarzenbach et al., 2010, 2006). These organic compounds and their metabolites are frequently detected at trace levels in fresh water environments and are therefore referred to as organic micropollutants. Organic micropollutants include a wide array of different compounds such as pesticides, herbicides, pharmaceuticals, food additives, and personal care products (Bao et al., 2012; Lapworth et al., 2012; Murray et al., 2010; Pal et al., 2010). With rapid advances in analytical techniques, new micropollutants have been discovered in the environment at a fast pace, causing increasing environmental concern (Carlson et al., 2013; Imfeld and Vuilleumier, 2012; Murray et al., 2010; Pal et al., 2010; Vorkamp et al., 2014). The assessment of the fate of these chemicals in the environment requires knowledge and information on their degradation pathways. However, the conventional approach based on concentration measurements of parent compounds and transformation products is often not conclusive, since a decrease of concentration might also be caused by dilution, sorption and further transformation of metabolites (Durst et al., 2013; Imfeld et al., 2013; Pal et al., 2010). Compound specific isotope

analysis (CSIA) measures changes in stable isotope ratios of different elements, and represents a valuable tool to study the transformation of various organic contaminants (Elsner, 2010; Elsner et al., 2005). Recent studies have applied CSIA to investigate the transformation of different organic micropollutants (Bashir et al., 2015; Elsayed et al., 2014; Zhang et al., 2014). Different reaction pathways have been characterized by analyzing dual-element stable isotope ratios of the parent compounds in an increasing number of experimental studies (Hartenbach et al., 2008; Maier et al., 2014; Meyer and Elsner, 2013; Penning et al., 2010; Reinnicke et al., 2011). Multi-element isotope data obtained from this experimental work provide valuable insights into the degradation mechanisms of organic micropollutants. Isotope models are useful tools to predict and quantitatively evaluate the isotopic evolution during degradation reactions, to link observed isotopic data to specific reaction mechanisms (Hofstetter et al., 2007; Hunkeler et al., 2009; Jin and Rolle, 2014) and to describe isotope fractionation in complex environmental systems where both physical and transformation processes may cause isotope fractionation and affect the observed isotopic signals (Eckert et al., 2012; Jin et al., 2014, 2013; Thullner et al., 2012; Van Breukelen and Rolle, 2012). To date, multi-element isotope modeling studies have focused on widespread groundwater organic contaminants such as chlorinated ethenes, MTBE and BTEX (Centler et al., 2013; Jin and Rolle, 2014). Although multi-element isotope modeling would be beneficial to evaluate isotope data also for different organic micropollutants, such an approach is still lacking for these contaminants. In particular, micropollutants are typically large molecules for which bulk isotope fractionation observed in environmental samples is often significantly diluted. For this reason, position-specific information, which goes beyond bulk isotope ratios typically addressed in CSIA applications, greatly helps to gain insights on the occurrence and on the mechanisms of specific degradation reactions. In this study we aim at presenting and

validating a multi-element isotope modeling approach that represents a valuable tool for the simultaneous quantitative interpretation of concentration as well as bulk and position-specific isotope data during the degradation of organic micropollutants. The approach builds on the foundations laid in a recent contribution (Jin and Rolle, 2014) that first proposed to incorporate mechanistic and position-specific understanding of contaminant degradation on model-based interpretation of isotope data. Model formulations are proposed in this study to simulate isotope fractionation during degradation of organic micropollutants. Such formulations are independent of commonly adopted linear regressions of dual-element isotope data. They are based on a mass conservation description of degradation reactions that allows incorporating mechanistic knowledge of isotopic evolution for different reaction pathways. The outcomes of the simulations highlight interesting and specific features characterizing micropollutants transformation and constitute a relevant improvement compared to commonly applied modeling approaches that rarely attempt to incorporate mechanistic information.

Specifically, the goals of this work are to: (i) present and illustrate multi-element isotope modeling approaches for degradation of organic micropollutants based on position-specific isotopologues which track atoms in positions experiencing isotope effects; (ii) apply the model to predict multi-element isotope changes during degradation of selected important organic micropollutants such as dichlorobenzamide (BAM), isoproturon (IPU) and diclofenac (DCF); (iii) illustrate the capability of the model to describe position-specific isotope fractionation occurring at and close to reacting bonds, to capture both primary and secondary isotope effects and to explore their possible extent of variation consistently with observed bulk isotope data; (iv) directly validate the outcomes of the proposed position-specific isotope modeling with available position-specific isotope data; (v) show the potential of the model as predictive tool to explore

the applicability of CSIA for different elements and degradation scenarios for which an experimental investigation is not (yet) available.

Modeling Approach and Reaction Pathways

Biodegradation of organic micropollutants in the environment can follow different reaction pathways. Since micropollutants often have large and complex molecular structures and their biodegradation involves the cleavage of specific bonds, an efficient way to simulate multi-element isotope fractionation for these compounds is to track position-specific isotopologues, considering atoms at isotopically-sensitive positions. This method represents a convenient balance between a fully-integrated approach (i.e., considering all possible isotopologues) that has been proposed for small molecules such as chlorinated compounds (Jin et al., 2013) and common, simplified, formulations exclusively considering two virtual heavy and light species (e.g., Eckert et al., 2012) and preventing to capture specific characteristics of different degradation pathways. The proposed modeling framework allows incorporating mechanism-specific information in the simultaneous description of concentration and stable isotope evolution during micropollutants transformation. The key steps of the model formulation are to:

- hypothesize the degradation reaction mechanisms and identify the fractionating atoms. Important elements for this initial conceptualization step are the insights of experimental investigation, ab-initio calculations and expert judgment;
- define position-specific isotopologues, which are a small subset of all possible isotopologues, exclusively containing atoms located at positions experiencing isotope effects. To identify the position specific isotopologues we introduce a “string notation”, which, for a dual-isotope system involving two elements A and B, reads as: $[A_{p,s}-B_{p,s}]$,

where the subscripts p and s indicate primary and secondary isotopic positions and the dash represents a chemical bond that may connect the fractionating atoms A and B;

- derive the fractionation factors for primary and secondary isotope effects based on corresponding apparent kinetic isotope effects (AKIEs). The latter can be calculated by reported enrichment factors or by directly fitting the model to measured position-specific isotope data;
- track the concentration evolution of each position-specific isotopologue undergoing degradation according to a specified kinetic rate law;
- compute the changes of position-specific and bulk isotope ratios.

Degradation mechanisms and position-specific isotopologues. To illustrate the proposed modeling approach three examples of micropollutants degradation have been selected. These examples include aerobic degradation of dichlorobenzamide (BAM), biotic hydrolysis of isoproturon (IPU) and aerobic degradation and reductive dechlorination of diclofenac (DCF). The degradation pathways for these compounds are schematically illustrated in Figure 1.

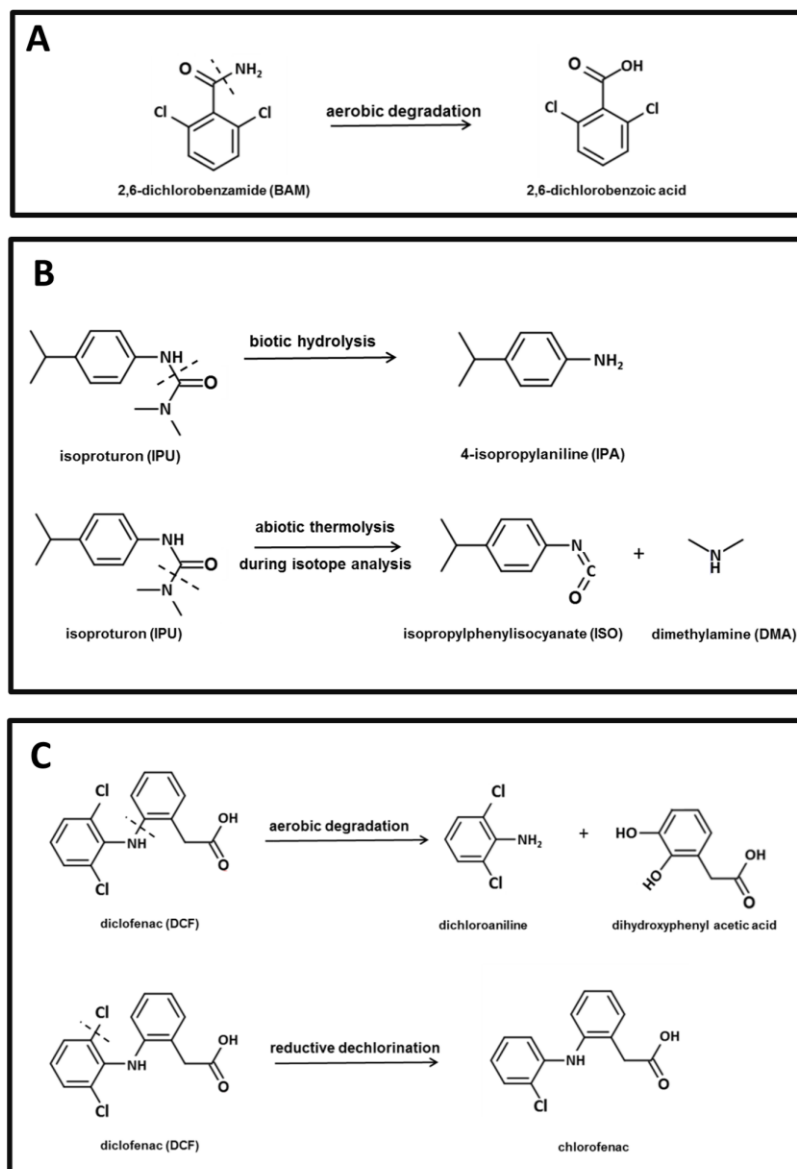


Figure 1. Reaction pathways for the selected organic micropollutants. Panel A: aerobic degradation of BAM; Panel B: biotic hydrolysis of IPU and abiotic thermolysis of IPU during compound-specific isotope analysis resulting in two fragments (isopropylphenylisocyanate, ISO, and dimethylamine, DMA) and; Panel C: suggested reaction pathways for aerobic degradation and reductive dechlorination of DCF.

134 Dichlorobenzamide is an important organic micropollutant. It is the main metabolite of the
135 herbicide 2,6-dichlobenil and, due to its mobility, it is often found in groundwater at
136 concentration levels higher than its parent compound (Holtze et al., 2008). Dual carbon and
137 nitrogen isotope ratios were measured to monitor microbial degradation of BAM (Reinnicke et
138 al., 2011). Significant carbon and nitrogen isotope fractionation were observed during BAM
139 degradation through microbial hydrolysis by two bacterial strains, *Aminobacter sp.* MSH1 and
140 *Aminobacter sp.* ASII. A nucleophilic substitution results in carbon and nitrogen isotope
141 fractionation at the carbonyl carbon as well as at the amide nitrogen. To model this reaction
142 pathway we consider the position-specific isotopologues, [C-N], to track the C and N atoms in
143 the reactive bond.

144 Isoproturon is used as systemic herbicide in cereal crops and it can be degraded by different
145 reaction pathways such as hydroxylation, N-demethylation and biotic hydrolysis. Among these
146 degradation pathways microbial hydrolysis of IPU is of special interest due to the fact that the
147 metabolite, 4-isopropylaniline, further reacts with soil organic matter, making this pathway
148 difficult to identify in aqueous environments (Barriuso et al., 2008). Biotic hydrolysis of
149 isoproturon by *Arthrobacter globiformis* D47 involves a complex reaction mechanism and C and
150 N isotope fractionation occurs at different parts of IPU molecules (Penning et al., 2010). This
151 reaction mechanism causes C and N isotope fractionation in different parts of IPU molecules.
152 Results from position-specific isotope analysis of the two thermolysis fragments of isoproturon,
153 isopropylphenylisocyanate (ISO) and dimethylamine (DMA), indicate that the carbonyl carbon
154 and the aryl nitrogen of ISO are at reactive and fractionating positions. The alkyl nitrogen and
155 the two methyl carbon atoms of DMA, instead, are at nonreactive positions but still undergo
156 fractionation due to secondary isotope effects (Penning et al., 2010). In order to track all the

atoms at positions experiencing isotope effects during biotic hydrolysis of isoproturon, we consider the position-specific isotopologues of isoproturon by tracking $[N_p-C_p C_s-N_s-C_s]$ (p : positions experiencing primary isotopic effects; s : positions experiencing secondary isotopic effects).

As last example of micropollutant degradation we consider the transformation of diclofenac, a widely used painkiller and anti-inflammatory agent. Diclofenac degradation occurs through both aerobic degradation and reductive dechlorination reactions. A very recent study successfully distinguished the two reaction pathways using dual-element C and N CSIA (Maier et al., 2014). and showed different extents of C and N isotope fractionation during aerobic degradation and reductive dechlorination. Assuming that a C-N bond is cleaved during aerobic degradation of diclofenac, our model tracks $[C-N]$ for the position-specific isotopologues of DCF. In the reductive dechlorination of diclofenac, instead, one C-Cl bond is cleaved, leading to primary carbon isotope effects at the C-Cl position and secondary nitrogen isotope effects at the neighboring nitrogen atom. Thus, for this reaction pathway the model tracks the isotopically-sensitive atoms $[N C-Cl]$. Furthermore, a modeling scenario has been carried out to account for possible secondary chlorine isotope fractionation occurring at the chlorine atom adjacent to the reactive C-N bond during aerobic degradation of diclofenac. Thus, the isotopically sensitive elements $[N-C Cl]$ have been considered in the simulation of 3D isotope fractionation, providing the simultaneous description of the three elements: C, N and Cl.

The illustrative examples presented in this study, the considered elements and stable isotopes, the reaction kinetics and the position-specific isotopologues used in the simulations are summarized in Table 1. The table shows the main characteristics of the modeling approach and reveals the rationale behind the selection of the illustrative examples. In fact, as summarized in the last

column of the table, each selected micropollutant particularly highlights a specific key feature of the proposed methodology.

Table 1. Specific features and information for the mechanistic modeling of the selected micropollutants.

Compound	Reaction kinetic	Isotope	Position-specific isotopologue	Key feature
Dichlorobenzimide (BAM)	Michaelis-Menten	$^{13}\text{C}/^{12}\text{C}$ $^{15}\text{N}/^{14}\text{N}$	$[\text{C}_s\text{-C}_p\text{-N}]$	Simulate primary and secondary isotope effects on atoms of the same element and their range of variation
Isoproturon (IPU)	first-order	$^{13}\text{C}/^{12}\text{C}$ $^{15}\text{N}/^{14}\text{N}$	$[\text{N}_p\text{-C}_p\text{ C}_s\text{-N}_s\text{-C}_s]$	Describe position-specific isotope fractionation and directly compare position-specific model predictions with position-specific isotope data
Diclofenac (DCF)	first-order	$^{13}\text{C}/^{12}\text{C}$ $^{15}\text{N}/^{14}\text{N}$ $^{37}\text{Cl}/^{35}\text{Cl}$	$[\text{N C-Cl}]$ $[\text{N-C Cl}]$	Explore three-dimensional isotopic evolution as well as predict isotope fractionation for an element (Cl) not yet experimentally investigated

Governing equations. To illustrate in detail the modeling approach, we select the complex case of biotic hydrolysis of isoproturon. Complete descriptions of the governing equations for the reaction pathways of BAM and DCF degradation are available in the supplementary material. As mentioned above, in order to model C and N isotope fractionation during biotic hydrolysis of isoproturon, the isotopically sensitive positions $[\text{N}_p\text{-C}_p\text{ C}_s\text{-N}_s\text{-C}_s]$ need to be tracked. Furthermore, the model considers the isotopic evolution of the two thermolysis fragments of IPU: isopropylphenylisocyanate (ISO) and dimethylamine (DMA), generated by position specific isotope analysis of isoproturon (Panel B in Figure 1). Since the occurrence of each nitrogen and

193 carbon atom of the j^{th} IPU isotopologue (with isotopically-sensitive atoms [N_p-C_p C_s-N_s-C_s]) is
 194 independent, the relative abundance of the j^{th} IPU isotopologue is given by the product of the
 195 abundance of each isotope:

$$A_j = X_{15N,p}^v \cdot X_{14N,p}^{1-v} \cdot X_{15N,s}^u \cdot X_{14N,s}^{1-u} \cdot Y_{13C,p}^i \cdot Y_{12C,p}^{1-i} \cdot Y_{13C,s}^h \cdot Y_{12C,s}^{2-h} \quad (1)$$

196
 197 where A_j is the relative abundance of the j^{th} position-specific isotopologue containing v ¹⁵N
 198 isotopes at reactive position (the subscript p represents primary isotopic positions) and u ¹⁵N at
 199 nonreactive position (the subscript s represents secondary isotopic positions) as well as i ¹³C
 200 isotopes at reactive positions and h ¹³C isotopes at nonreactive positions. X and Y are the
 201 abundances of N and C isotopes, respectively.

202 The carbon and nitrogen isotopes of isopropylphenylisocyanate (ISO) and dimethylamine
 203 (DMA) are fractionating according to the corresponding apparent kinetic isotope effects (AKIEs),
 204 which can be expressed as (Elsner et al., 2005):

$$\alpha_{C,p} = AKIE_{C,p}^{-1} \approx 1 + 10 \cdot \varepsilon_{C,ISO} \quad (2)$$

$$\alpha_{C,s} = AKIE_{C,s}^{-1} \approx 1 + \varepsilon_{C,DMA} \quad (3)$$

$$\alpha_{N,p} = AKIE_{N,p}^{-1} \approx 1 + \varepsilon_{N,ISO} \quad (4)$$

$$\alpha_{N,s} = AKIE_{N,s}^{-1} \approx 1 + \varepsilon_{N,DMA} \quad (5)$$

205 where α is the fractionation factor for primary or secondary isotope effects, ε is the bulk
 206 enrichment factor of ISO or DMA. The factor 10 in Eq. (2) is the ratio between the total number
 207 of carbon atoms and the number of the fractionating carbon atoms in ISO molecules. In the other
 208 equations this factor is 1 since all the atoms tracked experience isotope effects. Considering first-
 209 order kinetics, the reaction rate for the j^{th} [N_p-C_p C_s-N_s-C_s] isotopologue of IPU is given as:

$$r_j = -k \cdot (\alpha_{N,p})^v \cdot (\alpha_{N,s})^u \cdot (\alpha_{C,p})^i \cdot (\alpha_{C,s})^h \cdot C_j \quad (6)$$

210

211 where r_j is the reaction rate for the position-specific isotopologue, k is the first-order reaction rate
212 constant of the isotopologue containing only light isotopes, C_j is the concentration of the j^{th}
213 position-specific isotopologue and α is the fractionation factor defined in Eqs. (2-5).

214 The concentration change of isoproturon is described by tracking each position-specific
215 isotopologue:

$$\frac{dC_{IPU,j}}{dt} = r_j \quad (7)$$

216

217 where $C_{IPU,j}$ is the concentration of the j^{th} position-specific isotopologue of IPU, t is the time, and
218 r_j is the reaction rate of the j^{th} position-specific isotopologue. The total concentration of IPU is
219 obtained by summing the concentration of each isotopologue: $C_{IPU} = \sum_j C_{IPU,j}$.

220 The computed concentration of each position-specific isotopologue is used to calculate the
221 carbon and nitrogen isotope ratios of isopropylphenylisocyanate (ISO) and dimethylamine
222 (DMA) by counting the total number of heavy and light carbon and nitrogen isotopes,
223 respectively (Jin et al., 2011). Moreover, it is necessary to account for the dilution by the nine
224 non-fractionating carbon atoms in ISO, whereas the bulk nitrogen isotope ratios of ISO and
225 DMA are the same as the corresponding position-specific isotope ratios since there is no nitrogen
226 atom at non-fractionating position. Thus, the bulk isotope ratios for the two thermolysis products
227 of isoproturon can be computed as:

$$R_{C,ISO} = \frac{tot(^{13}C)}{tot(^{12}C)} = \frac{1}{10} \cdot \frac{\sum_{j=1}^n i \cdot C_j}{\sum_{j=1}^n (1-i) \cdot C_j} + \frac{9}{10} \cdot R_{C,0} \quad (8)$$

$$R_{C,DMA} = \frac{tot(^{13}C)}{tot(^{12}C)} = \frac{\sum_{j=1}^n h \cdot C_j}{\sum_{j=1}^n (1-h) \cdot C_j} \quad (9)$$

$$R_{N,ISO} = \frac{tot(^{15}N)}{tot(^{14}N)} = \frac{\sum_{j=1}^n v \cdot C_j}{\sum_{j=1}^n (1-v) \cdot C_j} \quad (10)$$

$$R_{N,DMA} = \frac{tot(^{15}N)}{tot(^{14}N)} = \frac{\sum_{j=1}^n u \cdot C_j}{\sum_{j=1}^n (1-u) \cdot C_j} \quad (11)$$

228 where R_C and R_N are the C and N isotope ratios of ISO or DMA, C_j is the concentration of the j^{th}
 229 position-specific isotopologue of IPU, DMA or ISO, $R_{0,C}$ is the initial carbon isotope ratio.
 230 Equation 8 for $R_{C,ISO}$ is given under the assumption that secondary isotope effects are only
 231 occurring in the DMA fragment. Since bulk isotope ratios are measured and reported in most
 232 isotope applications, our model can also predict bulk carbon and nitrogen isotope ratios of
 233 isoproturon. This is done by taking the weighted mean of the isotope ratios of both ISO and
 234 DMA:

$$R_{C,IPU} = \frac{10}{12} \cdot R_{C,ISO} + \frac{2}{12} \cdot R_{C,DMA} \quad (12)$$

$$R_{N,IPU} = \frac{1}{2} \cdot R_{N,ISO} + \frac{1}{2} \cdot R_{N,DMA} \quad (13)$$

235

where R_C and R_N are the bulk C and N isotope ratios of ISO, DMA or IPU, respectively. The weighting factors represent the number of carbon or nitrogen atoms in ISO or DMA fragments which are weighted by the total number of carbon and nitrogen atoms in IPU.

Results and Discussion

2,6-dichlorobenzamide (BAM) degradation. The results of the multi-element isotope modeling of BAM degradation are reported in Figure 2 where they are also compared with the experimental data of Reinnicke et al. (2012). The experimental study (Reinnicke et al., 2012) investigated biotic hydrolysis of diclorobenzamide by two microbial strains *Aminobacter sp.* MSH1 and *Aminobacter sp.* ASI1. The data (symbols in Fig. 2A) show a similar extent of C isotope fractionation for both strains (~22‰). However, strain MSH1 showed slightly stronger N isotope fractionation (-11.7 to 26.5‰) compared with ASI1 (-11.6 to 16.4‰). The solid lines in Fig. 2A represent the multi-element isotope modeling results, which accurately capture the observed C and N isotope fractionation. Similar but distinguishable dual-isotope plots are observed for both strains, suggesting similar transformation mechanisms for BAM biotic hydrolysis. The model implements the experimental observations suggesting that C and N isotope fractionation occurs due to the cleavage of the C-N bond and considers position-specific isotopologues of BAM by tracking the atoms contained in the reacting bond [C-N]. The outcomes of the model can be expressed as shifts both in bulk and position-specific isotope ratios. The latter are a direct result of the model, which simulates the cleavage of the C-N bond and thus the position-specific C and N isotope fractionation at the carbonyl carbon atom and the amide nitrogen atom, respectively. These results are reported in Fig. 2B and show that the variation in isotope ratios at the C-N position has the same extent as the bulk nitrogen isotope ratios, since there is only one nitrogen atom in BAM molecules. However, the shift of the undiluted position-

specific carbon isotope ratio is considerably larger than the observed bulk values and varies from
-26.3 to 139.9‰.

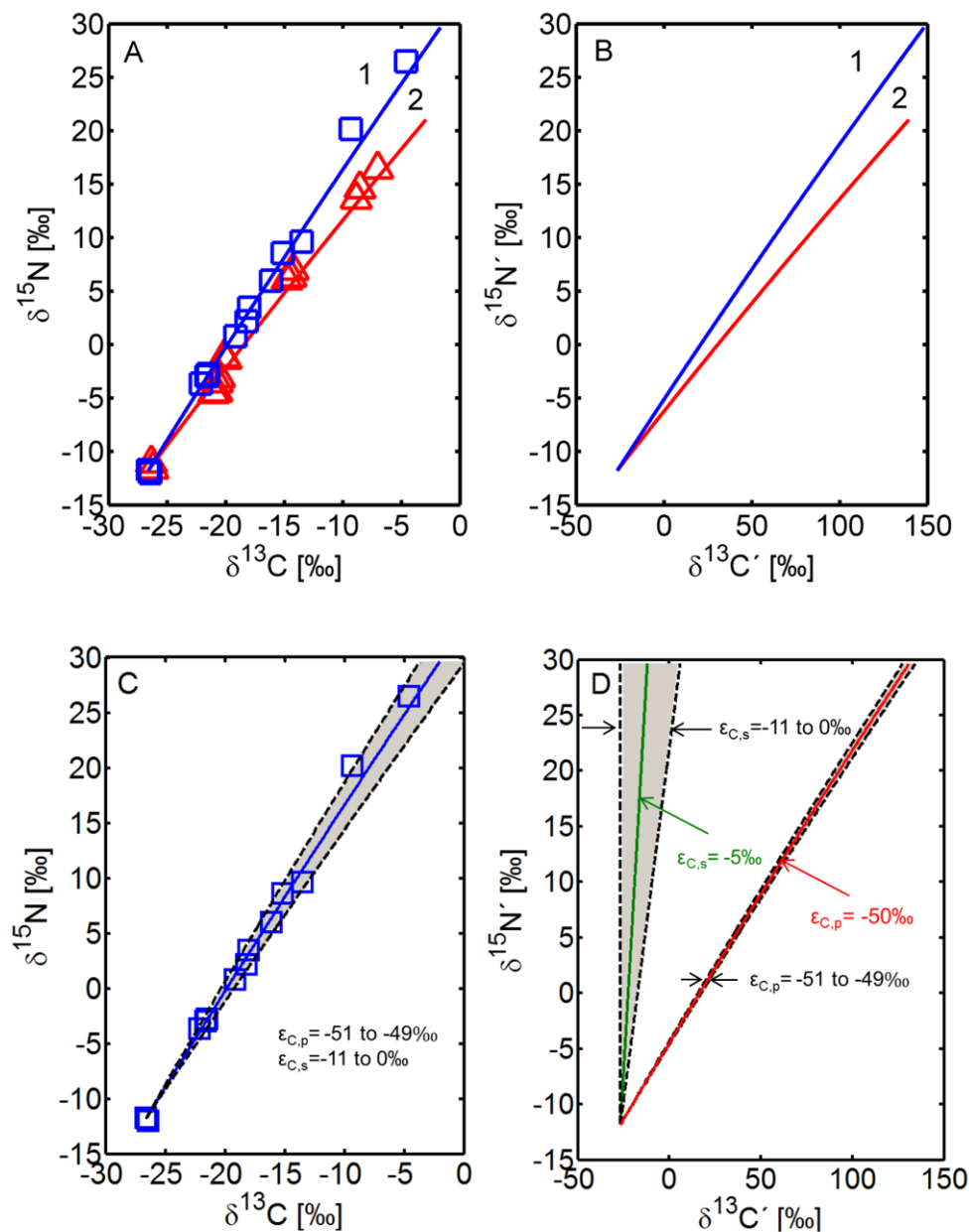


Figure 2. Carbon and nitrogen isotope fractionation during microbial hydrolysis of dichlorobenzamide (BAM). The symbols represent the experimental data reported by Reinnicke et al., 2012, and the solid lines are the simulation results. The numbers in Panels A and B indicate the degradation by two different microbial strains (1: *Aminobacter* sp. MSH1, 2: *Aminobacter* sp. ASI1). Panel B shows the position-specific C and N isotope fractionation at the carbonyl carbon atom and the amide nitrogen atom,

respectively. Panel C shows the simulation results for the scenario in which carbon isotope fractionation occurs on both C-N position and on the neighboring carbon atom; the dashed lines represent the range of variation of primary ($\epsilon'_{C,p}$ from -51 to -49‰) and secondary ($\epsilon'_{C,s}$ from -11 to 0‰) isotope effects consistent with the experimental data. The corresponding dual-isotope plots for primary and secondary isotope effects at the fractionating positions are reported in Panel D.

Besides illustrating our modeling approach for the interpretation of the experimental data for BAM degradation, we used numerical simulations to explore the magnitude of secondary isotope effects. Isotope fractionation due to secondary isotope effects has been observed during the degradation of different organic contaminants (Hofstetter et al., 2008; Kuder et al., 2013) but, to date, it has not been reported for BAM degradation. We performed simulations to estimate the effects of C isotope fractionation occurring at both reactive and nonreactive positions during BAM degradation. We considered the scenario in which both primary and secondary C isotope effects occur on the C-N position as well as on the neighboring carbon atom. The model formulation for BAM degradation was modified to include secondary isotope effects and to track $[C_s-C_p-N]$ isotopologues (p : position experiencing primary isotopic effects; s : position experiencing secondary isotopic effects). The simulation results for biotic hydrolysis of BAM by *Aminobacter sp.* MSH1 (reaction pathway 1) are reported in panel C and D of Figure 2. A number of simulations were performed spanning all possible combinations of primary and secondary isotope effects resulting in bulk C enrichment factors consistent and within the range of uncertainty of the experimental data (Fig. 2C). Isotope effects at fractionating positions are reported in Fig. 2D; the outcomes of the simulations based on possible values of primary and secondary enrichment factors are shown as shaded areas. The trends shown in Fig. 2D help visualizing the important contribution of both primary and secondary isotope effects at specific positions of BAM molecules. The primary carbon isotope fractionation at C-N position occurs according to an enrichment factor, $\epsilon'_{C,p}$, in a range between -51 and -49‰, which is similar and only slightly lower than the Streiwieser limit for cleavage of a C-N bond (Cook, 1991). The

position-specific enrichment factor, $\varepsilon'_{C,s}$, at the neighboring position, C_s , was varied from -11 to 0‰ to account for different possible extents of secondary isotope effects. The selected values of enrichment factors at C_p and C_s positions caused primary isotope fractionation varying from -26.6 to 134.3‰ as well as significant position-specific isotope fractionation at secondary isotope positions in a range between -26.6 and 6.0‰.

Isoproturon degradation. Multi-element isotope analysis has been used to investigate microbial hydrolysis of isoproturon (Penning et al., 2010). Such reaction mechanism yields different extents of carbon and nitrogen isotope fractionation. Furthermore, in that experimental study position-specific isotope analysis was performed to resolve isotope ratio changes in different parts of the isoproturon molecule (i.e. ISO and DMA fragments, Fig. 1). Following a common procedure, the position-specific enrichment factor and AKIE of IPU can be calculated from the measured bulk enrichment factor:

$$AKIE_{C,p}^{-1} \approx 1 + \varepsilon'_{C,IPU} = 1 + 12 \cdot \varepsilon_{C,IPU} \quad (14)$$

$$AKIE_{N,p}^{-1} \approx 1 + \varepsilon'_{N,IPU} = 1 + 2 \cdot \varepsilon_{N,IPU} \quad (15)$$

where ε' is the position-specific enrichment factor and ε is the bulk enrichment factor of isoproturon. The calculated values AKIE and ε' for IPU are reported in Table 2 together with the experimentally evaluated values for the fragments ISO and DMA. It can be noticed that IPU shows considerably higher position-specific enrichment factor and AKIE compared to the values directly determined for ISO and DMA. The reason of this inconsistency is that Eqs. 14 and 15 are based on the assumption that secondary isotope effects occurring at non-reactive position do not contribute to the overall bulk isotope fractionation. However, this is proved not to be the case for this degradation pathway, since isotope fractionation observed for DMA clearly shows the

occurrence of secondary isotope effects at the nonreactive positions. Therefore, we applied Eqs 2-5 to quantify separately primary and secondary isotope effects for both C and N. This results in more accurate AKIEs that are suitable to be used as model input parameters to simulate the isotope evolution during microbial hydrolysis of isoproturon. Alternatively, accurate position-specific AKIE values can be directly obtained with the proposed mechanistic modeling approach by fitting the model to the position-specific isotope data.

Table2. Evaluation of position-specific enrichment factors (ϵ') and AKIE values of IPU, ISO and DMA for biotic hydrolysis of isoproturon.

Compound	Isotope	n^\dagger	x^\ddagger	ϵ_{bulk}^* (‰)	ϵ' (‰)	AKIE
IPU	C	12	1	-5.3	-63.6	1.068
	N	2	1	-4.2	-8.4	1.008
ISO	C	10	1	-5.5	-55	1.058
	N	1	1	-3.7	-3.7	1.004
DMA	C	2	2	-1.8	-1.8	1.002
	N	1	1	-4.8	-4.8	1.005

[†]Number of atoms of the element considered

[‡]Number of atoms of the element located at fractionating position

* From data reported by Penning et al. (2010)

Numerical simulations were performed to illustrate the capability of the model to correctly reproduce the observed dual-element (C and N) isotope fractionation during IPU microbial hydrolysis. Moreover, the experimental dataset offers a unique opportunity to test the capability of the model to predict position-specific isotope fractionation since, as mentioned above, position-specific isotope effects were resolved for the two thermolysis fragments of isoproturon: isopropylphenylisocyanate (ISO) and dimethylamine (DMA). The simulation results and their comparison with the experimental data are shown in Fig. 3. The model is able to simultaneously reproduce the concentrations of isoproturon and its degradation product (4-isopropylaniline) as

well as the measured dual isotope data. The latter include the C and N isotope ratios observed for IPU as well as for the thermolysis fragments (ISO and DMA) produced during compound specific isotope analysis. Carbon and nitrogen isotope fractionation occurred at different extents for IPU, DMA and ISO and results in different trends and slopes in a dual-isotope plot (Fig. 3B). $\delta^{13}\text{C}$ values varied from -33.3 to -29.8‰ for ISO, -29.7 to -18.34‰ for DMA and -30.4 to -18.5‰ for IPU; $\delta^{15}\text{N}$ ranged from 0.3 to 13.1‰ for ISO, -5.0 to 3.8‰ for DMA and -7.4 to -2.6‰ for IPU. In particular, the different extents of C and N isotope fractionation observed for ISO and DMA fragments, clearly indicate that isotope fractionation involves the atoms at both reactive and nonreactive positions. Specifically, the cleavage of the bond between the carbonyl carbon and the alkyl nitrogen of IPU results in primary carbon and nitrogen isotope effects in ISO. Moreover, secondary isotope effects occur at the alkyl nitrogen and the two methyl carbons of DMA. Thus, C and N isotope fractionation of IPU is the sum of primary and secondary isotope effects occurring at ISO and DMA parts of IPU. Interestingly, the carbon isotope fractionation of DMA is only slightly smaller than the one of ISO, even though primary carbon isotope effects occur in the ISO fragment rather than in DMA. The reason for this effect is that carbon isotope fractionation occurring at the carbonyl carbon of ISO is diluted by the nine non-reactive carbon atoms in ISO. The outcomes of the mechanistic model are able to accurately reproduce the dual-isotope data of IPU, ISO and DMA. This substantiates that the proposed modeling approach correctly describes the reaction mechanism leading to the dual isotope evolution observed in the degradation experiments.

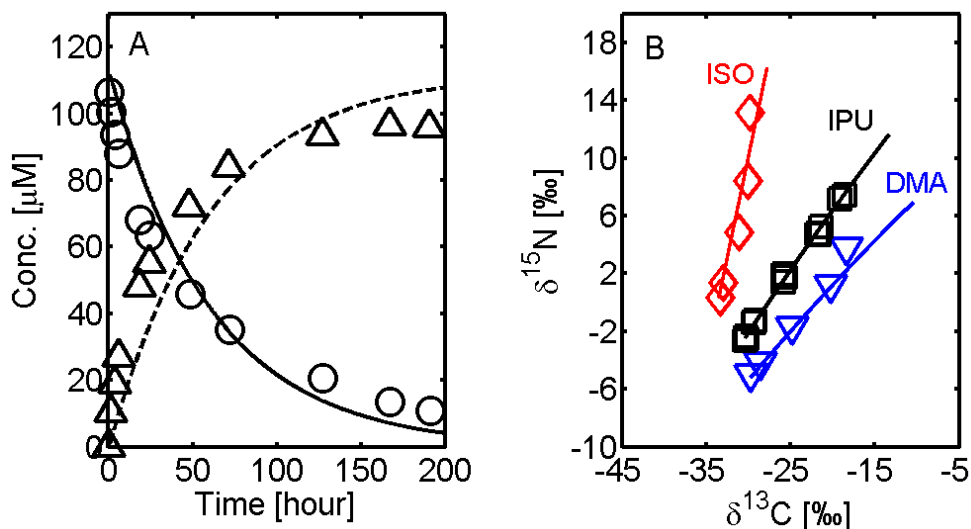


Figure 3. Concentration changes and carbon and nitrogen isotope fractionation during biotic hydrolysis of isoproturon (IPU). The symbols represent the experimental data reported by Penning et al., 2010, and the lines are the simulation results. In panel A the circles represent the concentration of IPU and triangles are the concentration of the hydrolysis product, 4-isopropylaniline. Panel B shows C and N isotope fractionation of IPU (squares) and the thermolysis fragments ISO (diamonds) and DMA (triangles).

Diclofenac (DCF) degradation. The third illustrative example focuses on diclofenac degradation. Both aerobic degradation and reductive dechlorination are important degradation pathways for diclofenac and have been found to yield significantly different extents of carbon and nitrogen isotope fractionation (Maier et al., 2014). To reproduce the isotopic data observed during DCF degradation, we simulated the carbon and nitrogen isotopic evolution during aerobic degradation (according to the suggested reaction pathway 1 reported in Fig. 4A) and reductive dechlorination (according to the reaction pathway 2 reported in Fig. 4A). The model (solid line in Fig. 4A) reproduces the experimental data (symbols in Fig. 4A), which are quite distinct for the two reaction mechanisms. In fact, aerobic degradation was accompanied by strong nitrogen isotope fractionation (-0.1 to 10.3‰), but only small carbon isotope fractionation (-26.5 to -25.0‰). Conversely, reductive dechlorination showed significant carbon but almost no nitrogen

isotope fractionation. As observed in the previous cases, the model also provides the position-specific fractionation of both C and N (Fig. 4B). The aerobic degradation involves isotope fractionation at C-N position, where $\delta^{15}\text{N}'$ shifted from -0.1 to -10.3‰ and $\delta^{13}\text{C}'$ varied from -26.5 to -18.4‰. Conversely, the cleavage of one C-Cl bond occurs during DCF reductive dechlorination, resulting in primary carbon isotope shifts at C-Cl position (-25.5 to 11.8‰) and secondary isotope shifts at the neighboring nitrogen atom (4.0 to 5.3‰). The two reaction pathways involve carbon atoms at two different positions, and different extents of corresponding position-specific carbon isotope fractionation are observed.

Reductive dechlorination of chlorinated organic contaminants involves the cleavage of C-Cl bonds; thus, Cl-CSIA is also of interest to elucidate the underlying reaction mechanisms and has been applied to investigate the degradation of different organic compounds such as chlorinated ethenes and ethanes (Cretnik et al., 2013; Hofstetter et al., 2007; Kuder et al., 2013; Palau et al., 2014). Even though no chlorine isotope data is available yet for diclofenac degradation, we performed simulations to predict the possible evolution of Cl isotopes. This scenario modeling was carried out to explore the potential of chlorine CSIA and required developing a 3D isotope modeling approach to simultaneously simulate C, N and Cl isotope fractionation. For the reactive dechlorination pathway, the bulk chlorine enrichment factors for DCF were selected in a range (-5.4 to -4.1‰) from the reported chlorine AKIE values observed during microbial reductive dechlorination of TCE (Cretnik et al., 2013). Concerning aerobic degradation of DCF, even if chlorine atoms are not at reactive positions, they might still fractionate due to secondary chlorine isotope effects. Since secondary chlorine isotope effects result in much smaller chlorine isotope fractionation, we used bulk chlorine isotope enrichment factor of -0.5 to -0.4‰ (estimated to be 1/10 of the primary chlorine isotope fractionation during reductive dechlorination of TCE) for

chlorine AKIE. As shown in Fig. 4C, the chlorine isotope signatures varied from 0 to 4.5‰ for reductive dechlorination but very small chlorine isotope fractionation, 0 to 0.45‰, was predicted for aerobic DCF degradation. The differences between the two reaction pathways predicted by the position-specific modeling scenarios are remarkable and indicate the potential also of chlorine CSIA to distinguish aerobic degradation and reductive dechlorination of DCF.

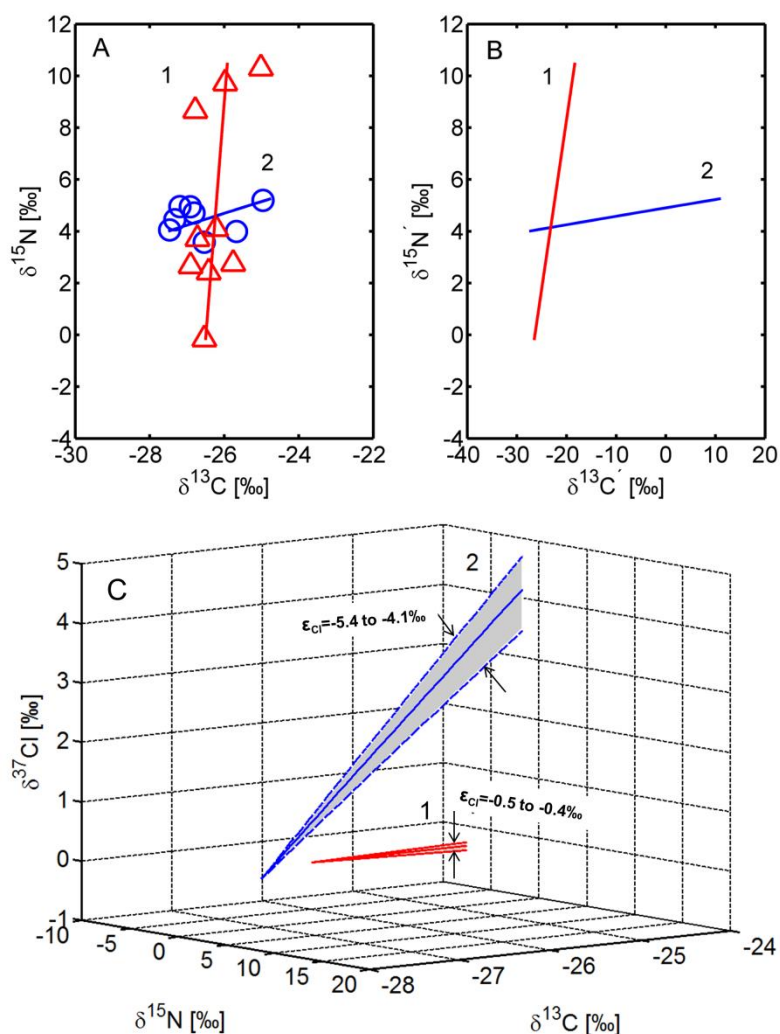


Figure 4. Carbon and nitrogen isotope fractionation during aerobic degradation (reaction pathway 1, red) and reductive dechlorination (reaction pathway 2, blue) of diclofenac (DCF). The symbols represent the experimental data reported by Maier et al., 2014 and the solid lines are the simulation results. Panel B shows the position-specific C isotope fractionation at C-N and C-Cl positions and the corresponding position-specific N isotope fractionation. Panel C shows the outcomes of the simulation scenarios using the 3D isotope modeling approach, also including Cl isotope fractionation.

Conclusions

Organic micropollutants have been increasingly detected in freshwater environments. Understanding the fate and transport of these trace organic contaminants remains a major challenge. Multi-element CSIA offers important advantages to characterize different reaction pathways and to elucidate reaction mechanisms of organic micropollutants degradation.

In this study we have presented a position-specific isotope modeling approach that is useful to quantitatively interpret multi-element isotopic evolution occurring during different transformation pathways of organic micropollutants. The proposed modeling approach simultaneously simulates concentration and multi-element isotope data and allows simulating both bulk and position-specific isotopic evolution. The approach has been validated with three illustrative examples of organic micropollutants degradation including 2,6-dichlorobenzamide, isoproturon and diclofenac. The simulation results could accurately capture the observed isotopic fractionation during the degradation of these micropollutants through different and fairly complex reaction pathways. Furthermore, the proposed modeling approach directly tracks isotopically-sensitive atoms in position-specific isotopologues and thus enables the possibility to simulate both position-specific and bulk isotope evolution. This capability is important since CSIA typically addresses changes of bulk isotope ratios, from which it is challenging to distinguish between primary and secondary isotope effects. As a result, secondary isotope effects are often neglected by assuming that only the cleavage of a chemical bond is contributing to the observed isotope fractionation. However, secondary isotope fractionation might be significant in particular for large organic molecules. Disregarding these effects might lead to an overestimation of AKIE values. To this end, the model represents a valuable instrument that allows considering

possible combinations of both primary and secondary isotope effects thus representing an important tool to evaluate experimental data and to explore and test different mechanistic scenarios. Another advantage, offered by the capability of the model to simulate position-specific isotope fractionation, is the possibility to directly compare the model outcomes with the results of position-specific isotope analysis, a technique that is likely to see an increasing number of applications due to the fast advances of analytical techniques (e.g. Breider and Hunkeler, 2011; Mckelvie et al., 2010; Wuerfel et al., 2013). Finally, besides the assistance in the quantitative interpretation of existing experimental observations, the model can be used to evaluate scenarios not yet explored experimentally as well as to design new experiments. For instance, this capability was illustrated in the case of biodegradation of diclofenac, for which predictive simulations of chlorine isotope fractionation have been performed. This will allow the fast screening of different experimental conditions and reaction mechanisms and may help in selecting the most promising experimental setups and isotope techniques to investigate important reaction pathways and contribute to advance the understanding of micropollutants degradation in different environmental systems.

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